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# Molecular Fragile X Screening in Normal Populations

**W. Christine Spence, Susan H. Black, Lee Fallon, Anne Maddalena, Emily Cummings, Gianna Menapace-Drew, David P. Bick, Gene Levinson, Joseph D. Schulman, and Patricia N. Howard-Peebles**

*Genetics & IVF Institute, Fairfax (W.C.S., S.H.B., L.F., A.M., E.C., G.M.-D., D.P.B., G.L., J.D.S., P.N.H.-P.); Department of Human Genetics, Medical College of Virginia, Richmond, Virginia (S.H.B., A.M., D.P.B., G.L., J.D.S., P.N.H.-P.)*

**In December, 1993, we initiated a pilot project in which DNA fragile X (fraX) testing was offered during routine prenatal or genetic counseling to all pregnant women seen at the Genetics & IVF Institute, most of whom were referred for the indication of advanced maternal age. A brochure on fragile X syndrome was sent to each patient prior to her appointment and was reviewed by a counselor or physician during the counseling session. As of June 1995, 3,345 patients were offered testing; 474 women with no identified family history of mental retardation or learning disability and 214 women with a positive family history accepted the test on a self-pay basis. The second population screened was 271 potential donors in our anonymous egg donor program. DNA from blood was tested by Southern blot using EcoRI/EagI and StB12.3. If an expansion was detected, CGG repeat number was determined by PCR-based analysis. Among the 474 patients with unremarkable family histories, three fraX carriers were identified (repeat sizes = 60+), whereas none were found in the 214 patients with a positive family history. Among the potential egg donors, two high borderline patients were identified (repeat sizes = between 50 and 59). Our ongoing study indicates that screening of pregnant or preconceptual populations for fraX carrier status using DNA testing is accepted by many patients and is an important addition to current medical practice.** 

**KEY WORDS: fragile X carrier screening, population screening, DNA fragile X testing, StB12.3 probe, CGG repeat number**

## INTRODUCTION

Since the availability of the StB12.3 probe for direct DNA analysis, fraX carrier screening has been offered to pregnant women seen in our Institute who have been identified with a suggestive family history [Black et al., 1992]. However, estimates of a female fraX premutation carrier frequency of 1/259 [Rousseau et al., 1993, 1995] prompted us to reexamine our criteria for offering fraX testing to our patients. Premutations may be passed silently through a family for many generations before enlarging to a length that could cause clinical consequences. It is likely based on the estimated frequency and inheritance pattern of the disorder that premutation fraX carriers may be found in families where there is no history of mental retardation (MR) or learning disabilities (LD).

Highly accurate and specific carrier and prenatal testing is available using Southern blot and PCR-based analysis to detect borderline, premutation, and full mutation expansions [Fu et al., 1991; Rousseau et al., 1991, 1992; Sutherland et al., 1991; Levinson et al., 1994; Maddalena et al., 1994]. Our center has comprehensive genetic diagnostic and testing services. In December 1993, we began offering fraX testing during routine prenatal or genetic counseling to all pregnant women seen in our Institute, regardless of whether a significant family history of MR, LD, or a related entity was present. Mandatory fraX testing of all potential donors in our anonymous egg donor program was also initiated at that time.

We report here the fraX carrier frequency observed from December 1993 through June 1995 in patient populations with and without a family history of MR or LD.

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Address reprint requests to W. Christine Spence, Genetics IVF Institute, 3022 Javier Road, Fairfax, VA 22031.

## METHODS

### Population

The patients seen in our Institute include primarily pregnant women referred for advanced maternal age. Patients are also seen for counseling prior to becoming pregnant. A brochure describing the fragile X syndrome and its inheritance pattern is sent to patients at the time the appointment is made. During the counseling session, a physician or counselor reviews the information furnished in the brochure and discusses the possibility of fragile X syndrome. FraX testing was offered to 3,345 patients on a voluntary, self-pay basis from December 1993 to June 1995 regardless of whether a suggestive family history was identified. Women who had relatives with MR, LD, autism, attention deficit, and/or language delay of unknown etiology consistent with X-linked inheritance were considered to have a positive family history. FraX testing was done on all egg donor candidates during this time period.

### Molecular Testing

EcoRI/EagI Southern blot analysis on blood samples was performed as described by Rousseau and colleagues [1991]. Both a short (5–7 hr) and long (26–30 hr) electrophoresis run were performed to ensure detection of very large, heterogenous smears as well as small expansions [Maddalena et al., 1994]. DNA samples with small or borderline expansions detected by the Southern blot were analyzed by a PCR-based test [Levinson et al., 1994] to obtain a more precise size.

### Prenatal Diagnosis

Fetal specimens on all pregnant patients requesting fraX testing were held in culture pending maternal test results. When a carrier was identified, the woman was offered prenatal testing. DNA fraX testing was performed on either the chorionic villus (CVS) cultures (two cases) or amniotic fluid (AF) cultures (one case) using the same method employed for blood samples [Maddalena et al., 1994]. Cytogenetic analysis of fetal cultures for detection of the FRAXA fragile sites was offered to all women identified as carriers.

### Cytogenetic Analysis

The method of induction of the fragile site in AF or CVS cultures was performed as described by Howard-Peebles and Maddalena [1992].

## RESULTS

Out of the total of 3,345 patients offered fraX testing between December 1993 and June 1995, 474 with no history and 214 with a positive family history requested the testing. Concurrent with this, fraX screening was initiated in our anonymous egg donor population, and 271 potential donors were tested.

In the patients without a family history who elected fraX testing, none of the 474 women had a full mutation. Three were found to have a premutation of 60 or more repeats (Table I). The repeat sizes were determined to be 60 ± 3, 64 ± 3, and 67 ± 3. These three women were counseled that this repeat had the potential to expand to a full mutation when passed to their children. All three had male fetuses that were tested, and in all three cases, the fetus received the mother's normal allele. Cytogenetic analysis of fetal cultures for detection of the FRAXA fragile sites was offered to the three carrier women identified. No fragile sites were detected on the X chromosome in either of the two cases, one CVS and one AF, that elected cytogenetic studies. Routine chromosome analysis was performed in all three fetal samples, and results were normal.

As shown in Table I, nine of the patients without a family history were determined to have allele sizes between 40 and 49 (low borderline). Alleles of this size have sometimes been referred to as being in the "grey zone" because they may or may not be unstable. These patients were counseled that they had an extremely low risk of having a child with the fragile X syndrome.

Of the 271 potential egg donors screened for fraX, two were determined to have an expanded allele measuring between 50 and 59 repeats (Table I). One woman had an allele that was measured at 52 ± 3 repeats, and the other had an allele of 55 ± 3 repeats. These two women were counseled that their risk of having a child with fragile X syndrome was low, but were offered prenatal testing when they became pregnant. Six of the egg donor candidates had allele sizes that were low borderline, between 40 and 49 repeats.

Of the patients tested with a positive family history, none was found with a full mutation or premutation. Two women were determined to have low borderline allele sizes (both were 40 ± 3 repeats).

## DISCUSSION

It has been estimated that the female premutation carrier frequency of fraX is approximately 1/259

TABLE I. Results of fraX Carrier Screening in 745 Women With No Family History of MR or LD

| Expansion size | Patients (n = 474) | Egg donors (n = 271) | Total frequency |
|---|---|---|---|
| Full mutation (200+ repeats) | 0 | 0 | 0/745 |
| Premutation (60-200 repeats) | 3 | 0 | 1/248 |
| 50-59 repeats | 0 | 2 | 1/373 |
| 40-49 repeats | 9 | 6 | 1/50 |

[Rousseau et al., 1993, 1995]. Thus, it appears that there may be a higher proportion of women who are fraX carriers and at risk for having an affected child than there are, for example, Caucasian couples who are at risk for having a child with cystic fibrosis (1/25 x 1/25 = 1/625 couples). Expansions near the FMR-1 gene account for most of fragile X cases, expansion to a full mutation only occurs when the enlarged allele is passed from mother to child, and there have been no reports of de novo expansions from normal to affected size [Yu et al., 1992; Snow et al., 1993]. Thus, virtually all couples at risk for having a child affected with the fragile X syndrome will be ascertained by testing only females.

DNA-based fraX testing is sensitive, accurate, and specific [Fu et al., 1991; Rousseau et al., 1991, 1992; Snow et al., 1993]. Accurate prenatal testing is available when the mother is found to be a carrier [Sutherland et al., 1991; Maddalena et al., 1994]. When EcoRI/EagI Southern blot analysis using long and short electrophoresis times is combined with PCR-based analysis, expansions ranging from borderline (as small as 30 extra bases, or 10 extra repeats) to very large heterogenous full mutations (>600 extra bases) can be reliably detected.

An integral component of any genetic test is the availability of appropriate genetic counseling, both to explain the test that is being offered and to counsel those with positive test results. In the case of fraX testing, those found to be carriers of a full mutation or premutation allele need to be counseled to discuss prenatal testing and potential relatives who are at risk. Those with a borderline allele size need counseling for reassurance that their children have an extremely low risk for having fragile X syndrome, but there may be potential implications for future generations or in other branches of the family. However, the majority of patients will have negative test results and the counseling will be straightforward. When such counseling is available, as it is in our specialized genetics center, offering fraX testing to all patients has proven to be a realistic and readily attainable option.

Three carrier women among 474 patients with no family history were identified. In addition, at-risk relatives of these patients have been identified, and are planning to be tested. The detection of carrier or affected individuals often leads to the identification of other positive or at-risk family members. In families with a negative history, carriers might otherwise not be ascertained until an affected child is born.

The acceptance rate among our patients, both with and without a family history, is considerable (total of 688/3,345 or 21%). Although the number of women tested to date is too small to be statistically significant, the substantial frequency of fraX carriers (1/248 with 60+ repeats) found among women in our patient population appears to confirm the high frequency of fraX carriers previously estimated in the general population. The detection of these three fraX carriers out of 745 women with no identifiable family history of MR or LD also illustrates the significance of making fraX testing available to all pregnant or preconceptual women.

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